Guide to Completing
The National Institute of Neurological Disorders and Stroke
&
The National Institute of Deafness and Other Communication Disorders
Animal Study Protocol Form
(*NINDS/NIDCD ASP)

[*Appendix 1]

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Appendices

Introduction

The purpose of this guide is to help both new and seasoned investigators navigate the complex and sometimes onerous task of completing their animal study protocol forms. This is now done electronically at https://webapp.nhlbi.nih.gov/ninds-iaspapp/iasp.asp. The first part of this guide duplicates the electronic forms and provides help on completing each section. Don't be put of by the length of the guide; the first section covering protocol completion is the most important. The remainder of the guide contains appendices, sample entries, and other information that you will find useful.

Where possible, we have provided an explanation of terms and the numerous acronyms you will encounter when completing the forms. In the first part of the guide, we list the training necessary for Principal Investigators and other personnel on the proposal. If you have any questions or comments about the guide, or suggestions for clarification or improvement, please relay them to the Animal Health and Care Section Office at (301) 496-2207 or (301) 496-2569.

I. Section A – Administrative Data

Investigator requirements:

a. To become a **P**rincipal **I**nvestigator (PI) of an animal study protocol an NINDS scientist (not applicable to NIDCD researchers) must be one of the following: tenured, on tenure track, a Staff Scientist, or a Staff Clinician. This is NINDS policy and not **A**nimal **C**are and **U**se **C**ommittee (ACUC) policy.

b. When an investigator other than a PI plays a major role in the preparation and execution of a protocol, the ACUC recommends the designation of a **C**o-**P**rincipal **I**nvestigator (Co-PI). The PI, however, is ultimately responsible for any work done under his/her protocol.

c. All investigators listed in the study must fulfill training requirements.

- **General Requirements**

  - **Principal Investigators and Co-Principal Investigators must –**

    a) complete a lecture course on *Guidelines for Principal Investigators* conducted by the **O**ffice of **A**nimal **C**are and **U**se (OACU). Registration information, schedules, and all other information can be found on the Office of Animal Care and Use training page – http://oacu.od.nih.gov/training/index.htm.

    complete a web-based refresher course every three years. It is accessed using the same link as above.

    b) enroll in the **A**nimal **E**xposure **S**urveillance **P**rogram (AESP) with **D**ivision of **O**ccupational **H**ealth and **S**afety (DOHS). It may be necessary to call DOHS first for an appointment. See information on the OMS page - http://dohs.ors.od.nih.gov/med_surveillance.htm.

  - **All other Co-Investigators (typically include post-docs, students, technicians, etc.) must –**

    a) complete the *Guidelines for Animal Users* conducted by the OACU. The refresher for this course must be taken every three years. Both the initial and refresher courses are web based. See information on the OACU web page – http://oacu.od.nih.gov/training/index.htm.

    b) enroll in the Animal Exposure Surveillance Program with DOHS. To enroll in this program call DOHS first to make an appointment. See information at - http://dohs.ors.od.nih.gov/med_surveillance.htm.

- **Additional Requirements Applicable to the Protocol Procedures –**

  - Training on using aseptic techniques is required for **all** investigators who are listed on a protocol that has a survival surgery procedure (see policy at http://ahcs.ninds.nih.gov/ACUC_pages/pg.html). Survival surgery refers to surgery performed on an animal that subsequently recovers from anesthesia. This training is conducted by NINDS/NIDCD vets [Registration, schedule, and contact information can be found at the following link - http://ahcs.ninds.nih.gov/acuc/a_training.html.] The ACUC can grant exemption from taking the course if an investigator listed on a survival surgery protocol will not perform the surgery. Please note that the PI requests an exemption.

  - If the protocol uses non-human primates (NHP), investigators must complete *Working Safely with NHPs*. NINDS/NIDCD vets conduct this course. Please call the **A**nimal **H**ealth and **C**are **S**ection (AHCS) office at (301) 496-2207 or (301) 496-2569 to schedule an appointment.

  - The Division of Occupational Health and Safety requires the completion of training in the safe handling of non-human primate tissue that may be contaminated with Blood Borne Pathogens (BBP). Please contact DOHS at (301) 496-2346 for more information.

  - If the study requires euthanizing animals using cervical dislocation or guillotine without anesthesia, proficiency must be certified by NINDS/NIDCD veterinarians (see policy at http://ahcs.ninds.nih.gov/ACUC_pages/pg.html). Please call the AHCS office at (301) 496-2207 or (301) 496-2569 to schedule an appointment.

  - Training in other protocol procedures must be indicated by the PI on the Investigator Training and Education Form (see http://ahcs.ninds.nih.gov/ACUC_pages/forms.html). NINDS/ NIDCD veterinarians will train or supervise training when needed.

II. Section B – Animal Requirements

1. **Number of Years**
   The ACUC recommends requesting for three years instead of 1 or 2 unless the PI is certain that the study will be concluded within a year or two.

2. **Protocol Type**
   a. There are three types of animal study protocols - *in vivo, in vitro*, and breeding. Any combination of these can be selected depending on the requirements of the study.

b. *In vivo* protocol – a protocol in which procedures are done on live animals. All the *in vivo* procedures must be schematically represented in a flow chart (see Appendix 2 for examples of a flow chart).

c. *In vitro* protocol – a protocol in which procedures or tissue removal is performed on an animal *after* euthanasia. Requested animal numbers have to be justified using an *in vitro* table or chart (see ACUC policy and an example at http://ahcs.ninds.nih.gov/ACUC_pages/pg_invitro.html).

d. Breeding protocol – a protocol in which animals are maintained and bred. It should be linked to a protocol defining the experimental use of the animals. A breeding chart must be used to justify requested animal numbers (see ACUC policy and examples at http://ahcs.ninds.nih.gov/ACUC_pages/pg_breeding.html).

e. If the protocol includes breeding, *in vivo*, and *in vitro* type procedures, all three - an *in vivo* flow chart, a breeding chart, and an *in vitro* table - should be attached. The charts/tables should be referenced in Section E.3 and Section F.

3. **Animal Numbers**

a. Animal numbers should be entered for each year as required by the study. However, animal usage in a given year is not restricted to the number specified for that year in the protocol. Provided the total number of animals is not exceeded, investigators may use animals throughout the duration of the protocol as needed. An amendment is required if the need exceeds the total number approved for the study.

b. For rodents, only weaned animals (older than 21 days) are counted.

c. Pre-existing animals must be counted if the protocol is a renewal. Pre-existing animals are animals that will be transferred from the expiring protocol to the renewal. Animals that are ordered or those that are physically housed under the expiring protocol are considered as pre-existing.

d. The animal numbers requested in this section have to match to animal numbers in other sections as follows:
   - They must match with the number in Section E.3 where justification for animal number is given.
   - They must match with the numbers in Section H assigned to United States Department of Agriculture (USDA) categories.
   - Depending on the type of protocol – the animal numbers in Section B must be consistent with the animal numbers in:
     - the flow chart for *in vivo* type,
     - the *in vitro* table for *in vitro* type, and
     - the breeding chart for breeding type.

4. **Species Data**

a. All species should be listed separately. It is advisable to list strains, especially when animals are immunocompromised or have other problems needing special attention/support of the AHCS.

b. The age range or weight of the animals should be specified, e.g. lactating mother with litter, juveniles, adults, pregnant mother, etc.

c. The source should be specified – vendor, in house, or specific information on importation from collaborators. If animals need to be imported or exported into or from NIH, specific procedures must be followed. Information on import and export procedures can be obtained at http://ahcs.ninds.nih.gov/AHCS_pages/index.html.

d. The holding and procedure locations should be specified. This information (number of cages by species and where the animals can be housed - Bldg and room number) can be obtained from the AHCS office by calling (301) 496-2207. Procedure room locations (if different from the PI laboratory) can also be obtained from the AHCS office.

Note: *Cage allocation is not under the purview of the ACUC. You can find information on cage allocation from your Branch/Section chief or the Scientific Director.*

e. PIs need to be aware of animal ordering procedures at NIH. The instructions can be found at http://ahcs.ninds.nih.gov/AHCS_pages/index.html.

III. Section C - Transportation

NIH transportation guidelines must be followed – see http://oacu.od.nih.gov/ARAC/transport.pdf. Animal holding facilities may have additional transportation requirements according to the building where the animal holding facility is located (see http://ahcs.ninds.nih.gov/ACUC_pages/pg_trans_guide.html).

Sample description:
"Transportation of animals will follow NIH transportation guidelines. All transportation of mice between the Building 35 SAF and Building 10NMR Center will be in NIH-approved disposable cardboard transport boxes with filter paper covering all openings/air vents. Rats will be transported from Building 35 SAF to the Clinical Center using the NIH approved disposable cardboard transport boxes. The Clinical Center Animal Transportation Policy will be followed, including use of only the designated "animal only" elevator (Elevator# 28) to transport the animals between floors."

IV. Section D – Study Objectives

1. Objectives must be presented in terminology understandable to a lay person. Non-affiliated and non-scientific members of the ACUC should be able to understand the objectives.

2. The benefit of the study to human/animal health and/or to basic scientific knowledge must be described.

3. The goals of the study should be clearly defined and enumerated (if there is more than one goal) and linked to the appropriate experimental procedures (see examples in Appendix 3).

V. Section E – Rationale for Animal Use

1. Rationale for animal use – why use animals and not non-animal models, such as cell lines, computer models, etc.?

2. Appropriateness of species – the justification for using the selected species and no other. If more than one species, justification for each species must be provided separately.

3. Justification for requested animal number – there should be a justification for the requested animal number and not simply a list of animal numbers. If multiple species are used, then justify the numbers for each species separately.

    a. Justification of animal numbers for a **Breeding protocol** – the justification should be summarized in Section E.3 with details of the breeding scheme in the breeding chart (see http://ahcs.ninds.nih.gov/ACUC_pages/pg_breeding.html).

    b. Justification of animal numbers for an ***in vivo* protocol** – the justification should be fully described in this section. Justifications can be statistical (e.g. statistical power), based on published results or on prior experience.

    c. Justification of animal numbers for an ***in vitro* protocol** – justification should be based on requirements of each *in vitro* project planned under the protocol (see http://ahcs.ninds.nih.gov/ACUC_pages/pg_invitro.html).

VI. Section F – Description of Experimental Design and Animal Procedures

1. All procedures that will be performed on animals (living or dead) must be described in this section within the context of the experimental design. Procedures include:
    a. Live Animals
        - minor and major survival surgery

- multiple survival surgery (see policy - http://ahcs.ninds.nih.gov/ACUC_pages/pg_mmss.html)
- non-survival surgery
- behavioral or other testing
- blood sampling
- administering chemicals, drugs, radioactive materials, biological agents, DNA
- genotyping (see policy – http://ahcs.ninds.nih.gov/ACUC_pages/pg_tailsnip.html.
- identification methods (see policy - http://ahcs.ninds.nih.gov/ACUC_pages/pg_tailsnip.html
- imaging (MRI, CT, PET) - If animals will undergo MRI procedures, PI should complete an NINDS NMR form (see http://ahcs.ninds.nih.gov/ACUC_pages/forms.html), and the protocol should be reviewed by the NMR Center or the Mouse Imaging Facility (MIF). Contact the NMR/MIF for information at (301) 594-3898.
- irradiation
- animal restraint methods
- *in vitro* experiments (brief discussion)
- etc.

b. Dead Animals
An animal study protocol is required to use tissue collected from still-born animals or animals that were euthanized under a different protocol.

2. Post-procedure care of animals, in consultation with NINDS/NIDCD vets (if needed), should be summarized in this section with specific instructions in an Intervention and Endpoints table (see below for information on endpoints). Information on signs and symptoms with intervention assessments and treatment measures or endpoints should be specified in this table (see example on http://ahcs.ninds.nih.gov/ACUC_pages/forms.html). This table is used as a reference by animal holding facility vets and other facility staff who provide care to the animals.

3. The description of survival surgical procedures should be brief in this section. A detailed description should be provided in Section G.

4. All non-survival surgeries should be fully described in this section, not in Section G. Non-survival surgery is defined as any surgery performed on an animal that is subsequently euthanized without having recovered from general anesthesia.

Examples include:
a. C-section to remove fetuses followed by euthanasia of the mother.
b. terminal electrophysiology, etc.

5. Description of blood withdrawal procedure is required, and the rationale for blood sampling. The description should distinguish between survival and terminal.

a. **Survival** – State whether sampling is single/serial, frequency of blood draw, blood volume, site of withdrawal, etc. (See Appendix 4, and ARAC guidelines at http://oacu.od.nih.gov/ARAC/survival.pdf.

b. **Terminal** – Cardiocentesis should be described in this section together with the anesthesia that will be used and how the depth of anesthesia will be ascertained (e.g. absence of withdrawal and blink reflexes).

6. **Radioisotopes** – a description of the dose and route of administration with how a radioactive material will be used, and who will administer it to animals must be provided.
   a. Investigator must complete the training required by Radiation Safety before using radioisotopes. For training information, please see the Radiation Safety web page at http://www.nih.gov/od/ors/ds/rsb/rso/index.html.
   b. Radiation Safety must review and approve the protocol (see also Section K). The name of the radiation safety personnel can be found at http://www.nih.gov/od/ors/ds/rsb/hp/index.html or by calling Division of Radiation Safety at (301) 496-5774.
   c. Radiation Safety **S**tandard **O**perating **P**rocedure (SOP) must be followed. Contact Radiation Safety by calling (301) 496-5774 for a copy of the SOP.
   d. If animals will be returned to the holding facility after a radiation procedure, the holding facility must be notified in advance about the use of radioactive materials.
   e. If using irradiators, the facility housing the irradiator should review and indicate its ability to support the protocol by signature. The location (Bldg. and room number) of the irradiator should be specified in Section B.

7. **Restraints** – any form of restraint should be described and the investigators involved should have the appropriate training. If training is needed, contact your veterinarian through the Animal Health and Care Section (AHCS) office at (301) 496-2207. Prolonged physical restraint must be justified scientifically and is considered an exception to policy. The method of restraint must also be described in Section M.
   a. restraint cannot be used as a method of housing animals, or for convenience.
   b. restraint is only used when it is an experimental requirement.
   c. the duration of restraint should be the minimum time required to meet the objective of the experiment.

8. **Drugs and Chemicals** - Drug and/or chemical classes (e.g. dopamine antagonists, glutamate agonists) should be stated together with the dose, volume, concentration, route of administration, vehicle, and side effects. The dose, volume, concentration, and route of administration should be appropriate for the selected species and drug/chemical. If you are unsure, contact your veterinarian through the Animal Health and Care Section (AHCS) office at (301) 496-2207. If more than one drug/chemical is used, the drugs/chemicals should be listed in a table with the appropriate details (concentration, route of administration, side effects etc. (See example in Appendix 5). This requirement is intended to help address potential animal welfare concerns.

9. **Experimental Endpoint Criteria** – all studies must specify the end point of an experiment. Typically this will be euthanasia at a predetermined time. It is important to distinguish *experimental* endpoints (time points at which *in vivo* data collection are completed) from those required for medical reasons such as unrelieved pain or distress. *Medical* endpoints should be detailed in the Intervention and Endpoints Table (see http://ahcs.ninds.nih.gov/ACUC_pages/forms.html). In some cases medical and experimental endpoints may overlap.

   If cardiac perfusion for fixation is the experimental endpoint, it should be described in this section and Section J. Sample description: Animals will be deeply anesthetized with a 5% isoflurane that is maintained at 2% throughout the procedure. Depth of anesthesia will be verified by pinching all four paws with forceps to make sure that there is no withdrawal reflex. The thorax will then be opened and animals will be transcardially perfused with 4% paraformaldehyde using aortic and right atrial cannulae in a closed system. The aorta (via left ventricle) and the right atrium will be cannulated so that a gravity-fed infusion of saline or PBS followed by paraformaldehyde perfuse the brain. The chemical waste will be collected as liquid waste per NIH waste disposal guidelines without being exposed to the atmosphere. Perfusion will be done in a chemical fume hood or on a down draft table. The person performing the procedure will wear a lab coat and gloves.

VII. Section G – Survival Surgery

Survival surgery is surgery performed on an animal that subsequently recovers from general anesthesia.

1. A description of all survival surgery procedures (minor, major) must be provided in G.1.
   a. A detailed description of the survival surgery procedure/s and the aseptic surgery techniques that must be followed in any survival surgery must be described. Contact NINDS/NIDCD veterinarians if you need any assistance.
   b. Non-survival procedures should be described in Section F **not** G.

2. Individuals performing the surgical procedure(s) must be identified in G.2. These individuals must be fully trained in the procedure/s as supported by the training and experience form. If any training is required contact your veterinarian through the AHCS office at (301) 496-2207. Individuals who will perform survival surgery must complete the NINDS Aseptic Surgical Techniques Training (see http://ahcs.ninds.nih.gov/acuc/a_training.html).

3. Identify the building and room where surgery will be performed together with Animal Health and Care Program requirements. Such program requirements include:
   a. Survival surgery on rodents can only be conducted on three days, Monday to Wednesday, unless special arrangements are made with AHCS veterinarians. This is necessary so that the recovery of the animals can be adequately monitored before the weekend.

b. For animals that have undergone surgery, their cages should be flagged with a watch card and a yellow surgery card completed for each cage when returned to their rooms after surgery. This is to identify them for follow up and monitoring.

c. The Principal Investigator should contact the facility veterinarian to learn the requirements for animals housed in non-NINDS managed facilities. For example, animals housed in Building 49 are managed by the National Eye Institute.

4. Post-operative care of animals and personnel who will provide this care must be specified in G.4.

5. If multiple survival surgeries are performed, the scientific justification should be presented in G.5 and G.6 (See policy - http://ahcs.ninds.nih.gov/ACUC_pages/pg_mmss.html).

VIII. Section H – Pain/Distress Category

1. All animals requested under a protocol must be assigned to applicable USDA pain/distress category/categories. There are three categories: **C, D** and **E**. Animals in categories D and E (see below) require a literature search to establish that no valid alternatives exist for any of the experimental procedures that involve the potential for more than momentary/transient pain or distress to the animals. At least two databases (e.g. Agricola, Medline) should be searched. Enter combination of key words into the search for which alternatives are being sought [e.g. 1) alternatives, pain, distress, C-section, mouse; 2) alternative, pain, distress, laminectomy, mouse; 3) alternatives, pain, distress, perfusion, rat; 4) alternative, pain, distress, thoracotomy; 5) alternative, cardiocentesis, pain, distress, mouse; etc.]. Provide a brief description of the search results. If alternatives are available but they are not appropriate for your study, state why they are not appropriate. The NIH library provides training and assistance in literature search for alternatives (see http://nihlibrary.nih.gov/). You may also contact Mr. Bradley Otterson (Biomedical Librarian, ottersob@ors.od.nih.gov) for assistance with searches for alternatives.

   a. **Column C** animals are those undergoing procedures that involve no, minimal or transient pain and/or distress. A search for alternatives is not required. Examples – all animals used for *in vitro* experiments are assigned to Column C since no procedure is performed before the animal is euthanized.

      Subcutaneous (SC), intra-peritoneal (IP), intravenous (IV), or intramuscular (IM) injections of drugs are examples of procedures that produce minimal/transient pain/distress. Also included in this Column is anesthesia administered for euthanasia, animal restraint or imaging.

   b. **Column D** animals are those that undergo procedures causing pain and/or distress, which is relieved using anesthetics, analgesics, sedatives, or tranquilizers administered during and/or following the procedures.
      - Examples – all surgery including survival and non-survival surgery: craniotomy, tail snip of mice ≥ 21days, etc.
      - A search for alternatives to the procedure causing pain/distress is required.

c. **Column E** animals are those that undergo procedures in which pain and/or distress is *unrelieved*, or animals that die as a direct result of experimental procedures before the experimental end point is reached.
   - Scientific justification is required. The justification must be presented in a Column E form (http://ahcs.ninds.nih.gov/ACUC_pages/forms.html). Column E animals are reported to the USDA.
   - The number of animals in Column E should be minimized.
   - A monitoring plan and an intervention and endpoints table is required.
   - Requires a search for alternative procedures (see Column D above).
   - Examples of studies that may result in Column E animals include those inducing **E**xperimental **A**utoimmune **E**ncephalomyelitis (EAE), seizure, cancer, or those that use death as an end point.

2. The animal numbers listed in this section should match with those listed in Section B, Section E.3, and on any additional documents such as *in vivo* Flow Charts, *in vitro* tables, or Breeding Charts.

IX. Section I – Anesthesia, Analgesia, Tranquilization

1. All anesthetics, analgesics, sedatives, tranquilizers should be described in this section. The dose/volume**,** concentration, and route of administration must be included in the description (refer to Appendix 6; please also contact a veterinarian if you need assistance). The preferred anesthetic for mice and rats is isoflurane. If this cannot be used for scientific or experimental reasons, please consult NINDS/NIDCD veterinarians. Barbiturates are not recommended for anesthesia due to prolonged recovery time. Topical anesthetics are recommended when using ear bars, at incision sites, etc.

2. The description provided in this section should match that of other sections, such as Section F or Section G which may contain references to anesthesia and analgesia. Gaseous anesthetics, such as isoflurane, must be listed in Section K as Hazardous Chemicals/Drugs.

X. Section J – Method of Euthanasia

Euthanasia is the act of inducing *humane death* in an animal. It should result in *rapid unconsciousness* followed by cardiac or respiratory arrest and ultimate loss of brain function. The technique should *minimize any stress or anxiety* experienced by the animal prior to unconsciousness (AVMA Panel on Euthanasia, 2000).

1. General Rules
   a. *Only trained personnel* should euthanize animals, using appropriate techniques, equipment, and agents. Some methods, such as cervical dislocation, warrant special training and verification of skill. This includes AHCS technical staff and individuals listed on the animal study protocol.
   b. Animals should be euthanized in a non-public area where animals are not housed.

2. When to Euthanize
   a. Define the endpoints (both medical and experimental) for the study. Any study that may result in pain or distress requires an Intervention and Endpoints Table (see example - http://ahcs.ninds.nih.gov/ACUC_pages/forms.html).
   b. If there is the potential for morbidity or death as an endpoint, please refer to the ARAC *Guidelines for Endpoints in Animal Study Proposals* (see http://oacu.od.nih.gov/ARAC/Endpoints.pdf).
   c. In tumor studies, animals should be euthanized if the tumor interferes with normal behavior, ulcerates, develops necrotic areas, or if it is not palpable but clinical signs such as weight loss, lethargy, or loss of appetite appear.
   d. According to the **A**nimal **W**elfare **A**ct (AWA), animals that would experience unrelieved pain or distress must be euthanized at the end of (or during, if necessary) the procedure. Any deviation must be scientifically justified and approved by the ACUC. Animals that experience unrelieved pain or distress are considered Column E requiring a Column E Explanation Form (http://ahcs.ninds.nih.gov/ACUC_pages/forms.html) and must be reported to the USDA.

3. How to Euthanize
   a. **P**ublic **H**ealth **S**ervice (PHS) Policy states that the method of euthanasia must be consistent with the *2000 Report of the AVMA Panel on Euthanasia.* The AVMA report lists methods appropriate for various species. Any deviation from the report must be scientifically justified and approved by the ACUC.
   b. An animal's death **must** be verified before disposing of the carcass. Using injectable anesthetics, inhalant anesthetics, or carbon dioxide as a primary agent of euthanasia for rodents *requires* the use of a secondary physical means of euthanasia. For example, after using CO_2 inhalant, physical methods such as: cervical dislocation, decapitation, or creation of bilateral pneumothoraces is required to ensure the humane death of the animal.

4. Chemical Agents Appropriate for Euthanasia
   a. Carbon dioxide (inhalant)
      1) See *Guidelines for Euthanasia of Rodents Using Carbon Dioxide* at http://oacu.od.nih.gov/ARAC/euthanasia.pdf and *Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates* at http://oacu.od.nih.gov/ARAC/euthmous.pdf.
      2) The only acceptable source of carbon dioxide is from a regulated compressed gas cylinder. The NIH requires the CO_2 source to be stated in the animal study protocol.
      3) A second, physical method of euthanasia such as cervical dislocation, decapitation, or creation of a bilateral pneumothorax should be used in order to ensure humane euthanasia.

b. Inhalant anesthetics (isoflurane, Halothane)
   1) Inhalant anesthetics are potentially dangerous to humans and should only be used in a collection/scavenging system approved by DOHS. These inhalants should also be listed as Hazardous agents in Section K of the Animal Study Proposal.
   2) Requires a second, physical method of euthanasia such as cervical dislocation, decapitation, or creation of a bilateral pneumothorax in order to ensure humane euthanasia.

c. Injectable Anesthetics (pentobarbital, ketamine/xylazine)
   1) Pentobarbital used in large animals (carnivores, ungulates, nonhuman primates) must be given intravenously and body parameters monitored (heart beat and withdrawal reflexes or respiration) until death is confirmed. Pentobarbital may be the most practical method for euthanizing non-human primates.
   2) Pentobarbital used in rodents is given via intraperitoneal injection and a second physical method of euthanasia must be performed to ensure euthanasia.
   3) Other anesthetic agents (ketamine/xylazine, chloral hydrate) are not suitable as euthanasia agents unless they are used in conjunction with a terminal procedure such as perfusion or cardiocentesis for tissue collection. In these cases, the animal must be deeply anesthetized (no withdrawal reflex, blink response, etc.) before perfusion or cardiocentesis.
   4) Avertin used alone or as an anesthetic agent for perfusion or cardiocentesis is not acceptable because it is a poor anesthetic with minimal analgesic properties. Avertin's use is controversial and generally used only as a method of restraint or as the anesthetic for embryo transfer -- a procedure that takes less than five minutes by skilled personnel.

5. Methods of Euthanasia Requiring Anesthesia or Narcotization
   a. Cervical Dislocation
      1) Appropriate for mice and rats ≥ P14 but less than 200 grams, while anesthetized.
      2) See *NINDS/NIDCD ACUC Policy on Cervical Dislocation* at http://ahcs.ninds.nih.gov/ACUC_pages/pg_cervdis.html.

   b. Decapitation
      1) See *NINDS/NIDCD ACUC Policy for Euthanasia of Mouse and Rat Fetuses and Neonates by Decapitation* at http://ahcs.ninds.nih.gov/ACUC_pages/pg_euthanasia_neonate.html.
      2) Neonates (< P14) and fetuses may be decapitated with a sharp, heavy pair of scissors.
      3) Juveniles and adults can be euthanized by guillotine
         (a) Note the location of the guillotine and log book (building and room).

(b) Follow the AHCS Standard Operating Procedure (SOP) 301 titled Maintenance of Guillotines (Appendix 7), and state that you will follow the SOP in Section J.

c. Cardiac Perfusion and/or Exsanguination
   1) Animals must be **deeply anesthetized**. Physical parameters must be monitored to insure depth of anesthesia and the procedure must be fully described.
   2) Aldehydes/picric acid used for perfusion must be listed in Section K.

6. Euthanasia without Anesthesia or Narcotization
   a. Cervical Dislocation
      1) Cervical dislocation without anesthesia is allowed for rat and mouse fetuses and neonates < P14.
      2) Cervical dislocation of rats and mice ≥ P14 without anesthesia is not allowed, unless;
         (a) There is scientific justification for performing the procedure without anesthesia.
         (b) The person performing this procedure should be named in Section J, and should also demonstrate his/her proficiency to NINDS/NIDCD veterinarian. If they are not deemed proficient, they must receive training until they are able to perform the procedure in a manner that is consistently humane. The protocol will not receive its final approval until the designated investigator is proficient in the technique.
      3) See *NINDS/NIDCD ACUC Policy on Cervical Dislocation* at http://ahcs.ninds.nih.gov/ACUC_pages/pg_cervdis.html.

   b. Decapitation
      1) Decapitation without anesthesia is allowed for rat and mouse fetuses and neonates < P14.
      2) Decapitation of rats and mice ≥ P14 without anesthesia is not allowed, unless:
         (a) There is scientific justification for performing the procedure without anesthesia; and
         (b) a statement acknowledging adherence to Maintenance of Guillotines (Appendix 7) is included.

XI. Section K – Hazardous Agents

1. **Radioactive Materials (Radionuclides)**
   a. All radioactive materials must be listed in this section.
   b. Protocols with radioactive materials must be submitted by the PI to the appropriate Radiation Safety personnel (health physicist) for review and approval. Radiation Safety assigns health physicists to conduct review of protocols that will use radioactive materials. Health physicists are assigned by building and floors where

radioactive materials are used. The name of the health physicist can be located at http://www.nih.gov/od/ors/ds/rsb/hp/index.html or by calling the Division of Radiation Safety at (301) 496-5774.

c. The protocol must be approved by radiation safety before its final approval by the ACUC.

2. **Biological Agents**

a. Potentially hazardous biological materials that will be transferred to live animals should be listed in this section (e.g. bacteria, pertussis toxin, botulinum toxin, tetrodotoxin, ricin, picrotoxin, human/nonhuman primate cells, etc).

b. If human pathogens, toxins, or human or nonhuman primate blood, body fluid, or tissues are to be used in the proposed animal study, the PI must first complete and submit a Human Pathogen Registration Document (HPRD), along with a draft copy of the Animal Study Proposal(ASP), for approval by the NIH Institutional Biosafety Committee (IBC). A copy of the HPRD form may be found http://www.nih.gov/od/ors/ds/forms/hprd.pdf. If you need assistance, please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346.

c. After receiving IBC approval of the HPRD, the biological agents and HPRD number(s), and the Animal Biosafety Level (ABSL) assigned by the IBC must be listed in Section K. Standard safety practices and procedures for each ABSL, which may be found in the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* booklet, are indicated by reference to an ABSL. Additional safety practices and procedures recommended by the NIH IBC, NIH Biosafety Officer, or the IC Safety Specialist or those safety practices and procedures which may need to be emphasized due to the nature of the research must be described in the designated space in Section K. Please also contact the NINDS/NIDCD Safety Officer at 301-496-2346 for more information on NIH approved safety practices.

3. **Hazardous Chemicals/Drugs**

a. All potentially hazardous chemicals or drugs (e.g. volatile or gaseous anesthetics, carcinogens, mutagens, teratogens) that will be used in the proposed animal study must be listed in Section K.

b. All applicable safety practices and procedures, equipment, personal protective equipment, and disposal methods required for work with the potentially hazardous chemicals or drugs must be described in the designated space in Section K.
Sample Descriptions:

- "Isoflurane will be used under a chemical fume hood (or scavenger system, or a local exhaust ventilation system) approved by the Division of Occupational Health and Safety."
- "Formaldehyde will be used under a chemical fume hood (or scavenger system, or a local exhaust ventilation system) approved by the Division of Occupational

Health and Safety. Formaldehyde will be collected and disposed of as chemical waste following NIH approved safety guidelines."

- "Carcasses and animal tissue will be disposed of in MPW boxes following NIH approved safety guidelines."

Note: Refer to the **NIH Chemical Hygiene Plan,** http://www.nih.gov/od/ors/ds/pubs/chp/chemhygplan03.pdf, for information on hazardous chemicals. Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for more information on approved safety practices.

c. The safety requirements for handling hazardous chemicals/drugs are described in the manufacturers **M**aterial **S**afety **D**ata **S**heets (MSDS). MSDS are required for hazardous chemicals (except for commonly used chemicals such as formaldehyde, gaseous anesthetics, etc.) and should be submitted with the animal study protocol. A list of searchable MSDS databases can be found at http://dohs.ors.od.nih.gov/material_safety_data.htm.

4. **Recombinant DNA (rDNA)**

a. When material containing rDNA is to be used in a proposed animal study, the work must be approved by the NIH Institutional Biosafety Committee (IBC). The PI must submit the following to the IBC:

1) A completed **R**ecombinant **D**NA (RD) form. This form can be found at: http://forms.nih.gov/adobe/misc/NH2690.PDF. Information on completing this form may be found at: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html and http://www.nih.gov/od/ors/ds/pubs/guide.htm Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for assistance.
2) map (s) of the rDNA constructs
3) a copy of the animal study proposal
4) a completed HPRD form if necessary. Please consult with the NINDS/NIDCD Safety Officer.

c. The IBC meets once a month on the first Wednesday of the month. All rDNA applications must be received by the Committee at least *one week* before the meeting.

d. After receiving the IBC approval, the rDNA number(s) and the Animal Biosafety Level assigned by the IBC must be stated in Section K. The Animal Biosafety Level assigned by the IBC may be found on the IBC- approved rDNA. All applicable safety practices and procedures, equipment, personal protective equipment, and disposal methods required for work with rDNA must be described in the designated space in Section K. Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for more information on approved safety practices.

XII. Section L – Biological Material

1. Any biological material other than human or nonhuman primate (NHP) should be listed in this section.

2. A biological material must be tested when the historical details of tissue, tumor cell lines, or culture conditions (origins of serum, fibroblast feeder cells, etc.), such as microbiological conditions of the animals or animals used to generate these biological products, is unknown. **Test results must be attached to the protocol.**

3. To prevent accidental exposure of animals (especially rodents) to an infectious agent that may have contaminated a biological product, these materials must be tested according to the NINDS/NIDCD ACUC Policy regarding MAP, RAP, and HAP testing of Tissue Cultures and Biologics (see policy - http://ahcs.ninds.nih.gov/ACUC_pages/pg_testing.html).

   Definition:

   a. MAP **M**ouse **A**ntibody **P**roduction Test
   b. RAP **R**at **A**ntibody **P**roduction Test
   c. HAP **H**amster **A**ntibody **P**roduction Test
   d. IMPACT **I**nfectious **M**icrobe **P**CR **A**mplification **T**est

   These products must be tested to protect both the animal colony and human health.

4. A web link to recommended testing service - http://www.radil.missouri.edu/info/index.asp, or Contact the AHCS office at (301) 496-2207.

5. The PI certifies that the material has been tested and is uncontaminated by *initialing* this section.

6. If a human or nonhuman primate tissue or cell line will be used, an approved Human Pathogen Registration Document (HRPD) must be attached to the protocol, and referenced in Section K not in Section L.

XIII. Section M – Special Concerns or Requirements

All exceptions to policy should be justified, and concerns and special requirements should be described in this section. This section is referenced by facility managers to learn of any special requirements involved in housing animals. All concerns should be described in this section to ensure that the facility staff knows what to do when situations arise. Examples include:

- Special feed (breeder chow, dough diets, mash feed, etc.)
- Food on cage floor (surgery, illness, etc.)
- Metabolic caging
- Single housing of animals
- Delayed weaning

- If the mother is pregnant (due to monogamous pairs, for example), pups *must* be weaned before the next litter arrives.

XIV. Section N – PI Certification

1. The PI signs in this section certifying that he/she is responsible for overseeing the work conducted under the protocol. Principal investigator responsibilities include:

   a. In Column D and E protocols, ensuring that no valid alternatives exist to the painful or distressful procedures.

   b. Ensuring the research is not unnecessarily duplicative.

   c. Verifying that personnel listed in Section A:
      - Are enrolled in the NIH AESP.
      - Have attended the "Using Animals in Intramural Research: Guidelines for Animal Users" and triennial refresher course.
      - Received training in the biology, handling and care of the appropriate species.
      - Are familiar with Aseptic Surgical Methods (if performing surgery)
      - Are trained in research and testing methods that limit the use of animals or minimize distress, as well as the proper use of anesthetics, analgesics, and tranquilizers.
      - Know how to report animal welfare concerns

   d. Obtaining ACUC approval ***before***:
      - Performing procedures with significant deviations from those described in the protocol.
      - Allowing new personnel to conduct procedures. Inform the ACUC when removing personnel, as well.

XV. Section O – Concurrences

1. The name and signature of the Laboratory/Branch Chief or the Scientific Director is required in this section, indicating approval of the study by the Institute. Normally, the signature of the Laboratory or Branch Chief is sufficient. However, if the PI is the Chief of a laboratory, branch, independent Unit, or independent Section, it is the Scientific Director who signs in this space.

2. The names of the current Safety Representative (if applicable), Radiation Safety Officer (if applicable), the animal holding facility location/s, facility manager/s, facility veterinarian/s, and the Attending Veterinarian must be entered in this section. The ACUC Coordinator will obtain the signatures after the ACUC reviews and approves the protocol. Contact the ACUC Coordinator by calling (301) 496-2207 for current names of the above personnel.

XVI. Section P – Final Approval

1. Name of the current ACUC Chairperson should be entered in this section.
2. The Chair signs after the Committee approves the protocol.
3. Contact the ACUC Coordinator by calling (301) 496-2207 for the name of the current ACUC Chairperson.

The NINDS/NIDCD Animal Study Proposal Form (ASP)

NATIONAL INSTITUTES OF HEALTH ANIMAL STUDY PROPOSAL
NINDS/NIDCD
(Adopted 03/00)(See NIH Manual 3040-2)

PROPOSAL#	
ACUC Review	
APPROVAL DATE	
EXPIRATION DATE	

A. ADMINISTRATIVE DATA:

Institute, Center, or Division: _____

Principal Investigator: _____ *E-Mail:*

Building: Room: Telephone: Fax:

Unit, Section, Laboratory, or Branch:

Project Title:

_____ *of Proposal*

List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., co-investigator(s)):

Name	AUPI	AUPI Refresher	AESP
No Co-Investigators			

B. ANIMAL REQUIREMENTS:

ASP Requested for: [ ] One Year [ ] Two Years [X] Three Years

Protocol Type: [ ] In Vitro ASP [ ] In Vivo ASP [ ] Breeding ASP

Comments:

Species Data

C. TRANSPORTATION:

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within the Clinical Center, also include the route and elevator(s) to be utilized.

D. STUDY OBJECTIVES:

Briefly explain in non-technical terms the aim of the study and how the study may benefit human or animal health or advance scientific understanding of biological process.

E. RATIONALE FOR ANIMAL USE:

1) Explain your rationale for animal use.

2) Justify the appropriateness of the species selected.

3) Justify the number of animals to be used. (Use additional sheets if necessary.)

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:

Briefly explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following: (Use additional sheets if necessary.)

- *Injections or Inoculations (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)*
- *Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)*
- *Non-Survival Surgical Procedures (provide details of survival surgical procedures in Section G.)*
- *Radiation (dosage and schedule)*
- *Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)*
- *Animal Identification Methods (e.g., earpunches/notches, ear tags, tattoos, collar, cage card, etc.)*
- *Other Procedures (e.g., survival studies, tail amputations, etc.)*
- *Resultant Effects, if any, the animals are expected to experience (e.g., pain or discomfort, ascites production, etc.)*
- *Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.*

G. SURVIVAL SURGERY - IF PROPOSED, COMPLETE THE FOLLOWING:

1. *Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.(Use additional sheets if necessary.)*
2. *Who will perform surgery and what are their qualifications and/or experience?*
3. *Where will surgery be performed: Building/Room:*
4. *Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:*
5. *Has major survival surgery been performed on any animal prior to being placed on this study? If yes, please justify: ____*
6. *Will more than one major survival surgery be performed on an animal while on this study? If yes, please justify: ____*

H. PAIN OR DISTRESS CATEGORY :

The ACUC is responsible for applying U.S. Government Principle IV. Contained in Appendix 3: "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." Check the appropriate category(ies) and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT.

Describe your consideration of alternatives to procedures Listed for Column D and E that may cause more than momentary or slight pain or distress to the animals, and your determination that alternatives were not available. [Note: Principal Investigators must certify in paragraph N.5. that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.] Delineate the methods and sources used in the search below. Database references must include databases (2 or more) searched, the date of the search, period covered, and keywords used:

No Databases Searched

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route and schedule of administration.

J. METHOD OF EUTHANASIA OR DISPOSITION AT END OF STUDY:

Indicate the proposed method, and if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Panel Report on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

K. HAZARDOUS AGENTS:

Use of hazardous agents requires the approval of an IC safety specialist. Registration Documents are required to be attached for the use of recombinant DNA or potential human pathogens may be attached at the discretion of the ACUC.

		List agents and registration document number (if applicable)
[___]	*1. Radionuclides*	
[___]	*2. Biological Agents*	
[___]	*3. Hazardous Chemicals/Drugs*	
[___]	*4. Recombinant DNA*	

Study conducted at Animal Biosafety Level

Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study. Use of volatile anesthetics requires a description of scavenging methods used. Also describe methods for removal of radioactive waste and, if applicable, the monitoring of radioactivity.

Additional safety considerations:

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.):

1. *Specify:*
2. *Source: Material Sterile or Attenuated? ____*
3. *If derived from rodents, has the material been MAP/RAP/HAP/PCR tested? _____ (Attach copy of results)*
4. *I certify that the MAP/RAP/HAP/PCR tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.*

_____ *Initials of Principal Investigator.*

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY:

List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. ***I certify that*** *I have attended an approved NIH investigator training course.*

   Year of Course Attendance Location

   Year(s) of Refresher Training:

2. ***I certify that*** *I have determined that the research proposed herein is not unnecessarily duplicative or previously reported research.*

3. ***I certify that*** *all individuals working on this proposal who have significant animal contact are participating in the NIH Animal Exposure Surveillance Program.*

4. ***I certify that*** *the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal have attended the course "Using Animals in Intramural Research: Guidelines for Animal Users" and will complete refresher training as required, and received training in the biology, handling, and care of the species; aseptic surgical methods and technologies (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); procedures for reporting animal welfare concerns.*

5. ***FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section H): I certify that*** *I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) as noted in paragraph H, and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not*

6. *I will obtain approval from the ACUC before initiating any significant changes in this study.*

Principal Investigator Signature: _____ *Date:* _____

O. CONCURRENCES: PROPOSAL NUMBER - _____

Laboratory/Branch Chief *certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a laboratory or branch chief.*

Signature	*Date*

Safety Representative *certification of review and concurrence. (Required of all studies utilizing hazardous agents.)*

Signature	*Date*

Radiation Safety Officer *certification of review and concurrence. (Required of all studies utilizing radioactive materials.)*

Signature	*Date*

Facility Manager *certification of resource capability in the indicated facility to support the proposed study.*

Facility	*Signature*	*Date*

Facility Veterinarian *certification of review.*

Protocol Number

Facility	*Signature*	*Date*

Attending Veterinarian *certification of review.*

	Signature	*Date*

P. FINAL APPROVAL

Certification of review and approval by the NINDS/NIDCD Animal Care and Use Committee Chairperson.

CHAIRPERSON

Signature	*Date*

Example Flow Chart for *In Vivo* Studies

EXAMPLE of *in vivo* FLOW CHART

ASP#:
Title:

Total Number of animals requested = 25

- Day 1: Animals will undergo "X" surgery
- Day 7: All animals will be tested in behavioral tests "X", "Y", "Z"..
- Day 14: I.P administration of drug "X" in 4 different dosages
    - 5 Groups (N = 5 animals/group)
        - Group A: 1.0 mg/kg
        - Group B: 1.4 mg/kg
        - Group C: 1.8 mg/kg
        - Group D: 2.0 mg/kg
        - Group E: vehicle only
- Drug "X" will be administered daily for 7 days (Days 14 to 20)
- Day 26: Animals will be euthanized. Following euthanasia, tissue will be collected for histology, immunocytochemistry, etc.

Examples of Section D: Objectives

D. STUDY OBJECTIVES:

Briefly explain in non-technical terms the aim of the study and how the study may benefit human or animal health or advance scientific understanding of biological process.

It is estimated that about 1 - 2% of the population suffer from epilepsy. For some patients, there is a great need for more effective medication to control seizures. Given recent disappointment in the efficacy of new anticonvulsants, the ketogenic diet has once again sparked the interest of investigators (Vining EPG. Clinical efficacy of the ketogenic diet. Epilepsy Res 1999;37:181-190). Most anticonvulsant drugs in current use were developed using various seizure paradigms in experimental animals (e.g., pentylenetetrazole or maximal electroshock models).

The ketogenic diet was described before these models were developed, and as such, might not be subject to the same limitations as many of antiepileptic drugs that were developed through conventional seizure paradigms. Ketogenic diet has been in clinical use for over 80 years. It uses large amounts of fat and low quantities of carbohydrates. The ketogenic diet was designed to mimic the starvation state. It does this, but only to a degree - calories are still provided on the diet in an alternative form (i.e. fats instead of carbohydrates). Growth still occurs while patients are on the ketogenic diet. The ketogenic diet acquired its name based on the formation of ketone bodies in patients on the diet. Fatty acids can be broken down to ketone bodies (acetoacetate, beta-hydroxybutyrate, acetone) in the liver in a series of enzymatic reactions. Ketone bodies then serve as an alternate fuel source in the human brain. In humans, the ketogenic diet has shown greater efficacy in young infants and children, when compared to adults (Vining EPG. Clinical efficacy of the ketogenic diet. Epilepsy Res 1999;37:181-190).

Like in humans, the ketogenic diet has been shown to attenuate seizures in a variety of experimental models of epilepsy (Stafstrom CE. Animal models of the ketogenic diet: what have we learned, what can we learn? Epilepsy Res 1999;37:241-259; Appleton DB, DeVivo, DC. An animal model for the ketogenic diet. Epilepsia 1974;15:211-217; Bough KJ, Eagles DA. A ketogenic diet increases the resistance to pentylenetetrazole-induced seizures in the rat. Epilepsia 1999;40:138-143). The optimal timing for achievement of ketosis in rodents is approximately 14-28 days on the diet (Appleton, et al., and DeVivo DC, Leckie MP, Ferrendelli JS, et al. Chronic ketosis and cerebral metabolism. Ann Neurol 1978;3:331-337) and younger animals respond to the ketogenic diet better than the older ones (Appleton, et al., and Bough KJ, Valiyil R, Han FT, et al. Seizure resistance is dependent upon age and calorie restriction in rats fed a ketogenic diet. Epilepsy Res 1999;35:21-28). Despite numerous demonstrations of the anticonvulsant efficacy of the ketogenic diet both in humans and experimental animals, the mechanism of action of the diet is not well understood. As reviewed by Schwartzkroin, it is likely that there may be several mechanisms by which the ketogenic diet produces its net anticonvulsant effect (Schwartzkroin PA, Mechanisms underlying the anti-epileptic efficacy of the ketogenic diet. Epilepsy Res 1999;37:171-180).

The mission of our laboratory is to better understand mechanisms involved in epilepsy

and to use this knowledge to develop more effective and safer therapeutic modalities. In this proposal, we hope to contribute to this effort by investigating pharmacological mechanisms in the anticonvulsant efficacy of the ketogenic diet.

Goal 1. Anticonvulsant Mechanisms of the Ketogenic Diet

In general, seizure activity and epilepsy result from imbalance between excitatory (e.g. glutamatergic, cholinergic) and inhibitory (e.g. GABAergic, adenosinergic) neurotransmitter systems in the brain. Therefore, these excitatory and inhibitory neurotransmitter systems are the main targets for epilepsy research and drug development.

Anticonvulsant efficacy of the ketogenic diet has been demonstrated in experimental seizure models that are relevant to GABA receptors (e.g. models of seizures induced by pentylenetetrazol or bicuculline; Schwartzkroin PA, Mechanisms underlying the anti-epileptic efficacy of the ketogenic diet. Epilepsy Res 1999;37:171-180.) Surprisingly, effects of the ketogenic diet on non-GABAergic neurotransmitter systems have not been studied. Given that anticonvulsant efficacy of the diet in models relevant to GABA receptors is, at best, very modest and thus does not fully explain clinical efficacy of the diet, we speculate that the ketogenetic diet may additionally affect non-GABAergic excitatory neurotransmitter systems. There is indirect evidence to suggest that metabolic changes produced by the ketogenic diet can affect inhibitory (mainly GABAergic) as much as excitatory neurotransmitter systems (Schwartzkroin et al., 1999; Yudkoff M, Daikhin Y, Nissim I, Lazarow A, and Nissim I (2004) Ketogenic diet, brain glutamate metabolism and seizure control. Prostaglandins Leukot.Essent.Fatty Acids 70:277-285.)

To test our prediction we intend to use receptor-specific compounds to obtain a quantitative measure of changes in different neurotransmitter systems produced by the ketogenic diet using seizures as the clinically relevant end point. Each of the compounds we intend to use has well characterized pharmacological properties. Likewise, seizures produced by each of these compounds can be related to the specific receptor. Aminophylline (adenosine receptor antagonist) will be used to test possible effect of the ketogenic diet on non-GABAergic inhibitory neurotransmission. Pilocarpine (muscarinic receptor agonist) and several convulsants selective for different glutamate receptors (NMDA - selective for NMDA receptors, AMPA - selective for AMPA receptors, kainic acid - selective for AMPA/kainate receptors, ATPA - selective for gluR5 kainate receptors) will be used to test possible effects of the ketogenic diet on excitatory neurotransmission. A 6-Hz model will be used to further characterize anticonvulsant mechanisms responsible for clinical efficacy of the ketogenic diet. Seizures induced in the 6-Hz model originate in the limbic system and this model is particularly selective for evaluating anticonvulsant effects of neuroactive steroids (see Goal 3.) Taken together, functional changes in different neurotransmitter systems produced by ketogenic diet will be evaluated by comparing seizure sensitivity to several convulsive stimuli of mice maintained on the ketogenic diet (test group) and on normal diet (control group.)

Goal 2. Anticonvulsant Mechanisms of ketone bodies (acetoacetate, beta-hydroxybutyrate, and acetone)

Excessive production and subsequent accumulation of ketone bodies (acetoacetate, beta-hydroxybutyrate, and acetone) represent the hallmark of the ketogenic diet. There is some evidence to suggest that ketone bodies that accumulate in the ketogenic diet are responsible for the anticonvulsant properties of the diet (Yudkoff M, Daikhin Y, Nissim I, Lazarow A, and Nissim I, 2004, Ketogenic diet, brain glutamate metabolism and seizure control. Prostaglandins Leukot.Essent.Fatty Acids 70:277-285.) Recent experimental studies have demonstrated anticonvulsant properties of some ketone bodies in several seizure models (Rho JM, Anderson GD, Donevan SD, and White HS (2002) Acetoacetate, acetone, and dibenzylamine (a contaminant in l-(+)-beta-hydroxybutyrate) exhibit direct anticonvulsant actions in vivo. Epilepsia 43:358-361; Likhodii SS, Serbanescu I, Cortez MA, Murphy P, Snead OC, III, and Burnham WM (2003) Anticonvulsant properties of acetone, a brain ketone elevated by the ketogenic diet. Ann.Neurol. 54:219-226.) Except for evidence that some of the ketone bodies have anticonvulsant properties, little is known about pharmacological mechanism(s) responsible for their anticonvulsant efficacy. Like in Goal 1, we intend to test the three ketone bodies that are present in the ketogenic diet for their ability to attenuate seizures induced by receptor-specific compounds. Testing the ketone bodies against seizures induced by compounds of well-characterized pharmacological actions should shed more light on the possible mechanism of action of the ketones. It is a common practice in epilepsy research and during the antiepileptic drug development process to test a compound of interest against a variety of convulsive stimuli to delineate the compound's pharmacological mechanism of action and clinical efficacy for specific type of epilepsy (Loscher W and Leppik IE (2002) Critical re-evaluation of previous preclinical strategies for the discovery and the development of new antiepileptic drugs. Epilepsy Research 50:17-20.) Taken together, acetoacetate, beta-hydroxybutyrate, and acetone will be tested against seizures induced by the same convulsive stimuli as described in Goal 1. Unlike in Goal 1, only naive mice (no exposure to ketogenic diet) will be used. The results obtained in Goal 1 and Goal 2 together will allow for pharmacological differentiation of the anticonvulsant effects produced by the ketogenic diet itself and by the ketone bodies. If experiments from Goal 1 and Goal 2 yield markedly different profiles we will conclude that ketone bodies are not solely responsible for the anticonvulsant efficacy of the diet and that there must be other factors involved.

Goal 3. Role of Neuroactive Steroids in Anticonvulsant Efficacy of the Ketogenic Diet.

Neuroactive steroids are synthesized from cholesterol in peripheral endocrine glands and in the central nervous system (CNS) as metabolites of progesterone or desoxycorticosterone. In contrast to steroid hormones that regulate gene transcription through interactions with intracellular receptors, neuroactive steroids can alter the excitability of membrane-bound receptors in the CNS. Neuroactive steroids are positive modulators of the ionotropic gamma-aminobutyric acid receptor (GABA-A R), which mediates the majority of inhibitory neurotransmission in the brain. Neuroactive steroids, positively modulating the GABA-A R, possess high anticonvulsant activity in a number of experimental seizure models (Kokate et al., 1994, 1996; Reddy and Rogawski, 2000).

Schwartzkroin mentioned the potential role of "neuroactive steroids" in the control of epilepsy with the ketogenic diet (Schwartzkroin PA, Mechanisms underlying the anti-epileptic efficacy of the ketogenic diet. Epilepsy Res 1999;37:171-180.) Further, it has been recently demonstrated that the ketogenic diet results in regional elevation of certain neuroactive steroids in the brain (Harney JP, Rhodes ME, Seaman S, et al. Effects of a ketogenic diet in brain neurosteroids. Soc Neurosci Abstracts 2004). Our lab has been intensively involved in studying roles of Neuroactive steroids in epilepsy. We would like to further study potential roles of neuroactive steroids in the anticonvulsant efficacy of the ketogenic diet. Specifically, we intend to study if production of endogenous Neuroactive steroids helps to control seizures in the ketogenic diet. We postulate that elevated levels of endogenous neuroactive steroids may contribute to the anticonvulsant efficacy of the ketogenic diet. To verify this hypothesis we intend to use finasteride and indomethacin to block the last two enzymes (5 alpha-reductase and 3 alpha hydroxysteroid oxidoreductase, respectively) involved in production of endogenous neuroactive steroids such as allopregnanolone and allotetrahydroxycorticosterone. We have previously demonstrated the reversal of stress-induced elevation in seizure threshold when synthesis of these endogenous neuroactive steroids was blocked by finasteride or indomethacin (Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA-A receptor function and seizure susceptibility. J Neurosci 2002;22:3795-3805). (Stress is known to elevate levels of endogenous neuroactive steroids.) If our hypothesis is correct we expect that finasteride and indomethacin will decrease the anticonvulsant efficacy of the ketogenic diet by reducing diet-elevated levels of endogenous neuroactive steroids. In this study, groups of mice will be maintained on the ketogenic diet (test groups) and on regular diet (control groups) starting at P21. After at least of 14 days of exposure to the respective diet, the animals will be injected with finasteride or indomethacin before receiving convulsive stimuli. Pentylenetetrazol and 6-Hz seizure tests will be used as they represent most appropriate tests for evaluating anticonvulsant effects of neuroactive steroids (Gasior M, Carter RB, Goldberg SR, and Witkin JM (1997) Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjunction with diazepam. J.Pharmacol.Exp.Ther. 282:543-553, Kaminski RM, Livingood MR, and Rogawski MA (2004) Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. Epilepsia 45:864-867.) Blood samples will be collected post-mortem in selected animals for analysis of plasma levels of endogenous neuroactive steroids.

Guide for Survival Blood Sampling

Guidelines for Survival Blood Sampling in Animals

These guidelines have been developed to assist investigators in their choice and application of survival blood sampling techniques while writing and conducting an Animal Study Proposal (ASP).

Factors for Consideration and Required Detailed ASP Information

a. Species - Various species have various estimated percentages of Total Blood Volume (TBV) based on Total Body Weight (TBW)

Examples:	Mouse	TBV estimated mean of 7.2% of the TBW
	Rabbit	TBV estimated mean of 5.6% of the TBW
	Rat	TBV estimated mean of 6.4% of the TBW
	Rhesus macaque	TBV estimated mean of 5.6% of the TBW
	Beagle Dog	TBV estimated mean of 8.5% of the TBW
	Guinea Pig	TBV estimated mean of 7.5% of the TBW
	Minipig	TBV estimated mean of 6.5% of the TBW
	Horse	TBV estimated mean of 8.5% of the TBW

b. Animal Size (Weight) - Various animals within a species have various TBWs which in turn effects TBV. TBV is calculated using the TBW (in kilograms or grams) multiplied by the species dependent TBV percentage with a straight conversion of weight to volume (ex. kilograms = liters and grams = milliliters).

c. Restraint or Sedation Techniques - ASP must include a full and detailed technical description and justification for restraint or sedation.

d. Sample Desired (serum, whole cells, plasma, etc.) - ASP must include a full and detailed justification of why you need to conduct blood sampling.

e. Sample Quality (i.e., level of required sterility and techniques dictated by that level)

f. Sampling Site - ASP must include a full and detailed description and listing of all possible techniques and sites, respectively, that would be appropriate for the species and your research goals. It is always best to list as many alternatives as possible, especially when you consider “Murphy’s Law.”

Blood Sampling Site Examples:

Dorsal/Ventral Tail or Central Pinna Artery

Retrobulbar venous plexus - ASP must include a procedural description of this technique that includes topical anesthesia and alternating eyes for multiple draws

Jugular, Saphenous, Lateral Tarsal, Sub-Lingual, Cephalic,
Femoral, Marginal Ear, and Lateral Tail Veins

Cardiocentesis, Cranial Vena Cava, and Drip Collection by Tail Tip Amputation (≤2mm)

g. Animal's Health Status - Consideration of the animal's health status should always be made prior to blood sampling to assess the procedure's possibly affect on the animal.

h. Phlebotomist's Experience & Expertise - ASP must include specific identity of qualified individuals with a description of their expertise.

i*. Sample Quantity & Sampling Frequency* - ASP must include a full and detailed description, justification, and time line for single, multiple, or serial blood sampling.

Generally, no more than 15% of the animal's blood volume should be removed at one time. Rapid sampling rates or sampling of greater than 15% of the TBV in one iteration could possibly result in circulatory shock resulting in adverse affects to the animal. If 10 -15% of the TBV is removed in one sampling, the same volume of warm physiologic saline or Lactated Ringer's should be given back to the animal intravenously at an appropriate rate or via clysis by subcutaneous injection.

Blood Sampling Volume Limits and Recovery Periods

Single Sampling		Multiple Sampling	
% circulatory blood volume removed	Approximate recovery period	% circulatory blood volume removed in24 h	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
15%	4 weeks	20%	3 weeks

Blood Sampling Calculations

General Equation

TBW X %TBW for species TBV X Desired % of TBV for Draw X one milliliter/gram (or one liter/kilogram) = Draw Volume in mls or liters

1. 10% TBV Single Sampling **Mouse,** 20 grams TBW — 20 grams X .072 (7.2%) X .1 (10%) X milliliter / gram = .144 mls or 144 microliters

2. 7.5% TBV Single Sampling **Rat,** 250 grams TBW — 250 grams X .064 (6.4%) X .075 (7.5%) X milliliter / gram = 1.2 mls or 1200 microliters

3. 5% TBV Single Sampling **Rabbit,** 4 kilograms TBW — 4 kgs X .056 (5.6%) X .05 (5%) X liter / kilogram = .0112 liters or 11.2 mls

3. 15% TBV Single Sampling **Rhesus macaque** 8 kilograms (kgs) TBW — 8 kgs X .056 (5.6%) X .15 (15%) X liter / kilogram = .0672 liters or 67.2 mls

5. 10% TBV Single Sampling **Horse,** 500 kgs TBW — 500 kgs X .085 (8.5%) X .10 (10%) X liter / kilogram = 4.25 liters

An Example of a Drug Table

EXAMPLE
Table of Experimental Drugs

Name	Concentration	Dose & Volume	Route of Administration		Side-effects
			Site	Route	
USPIO (Iron Oxide Particles)	~30-1000nm Diameter	10-400umol of iron/kg body weight; 1ml or less for IV < 1ul forstereotaxic	Rear Leg or Brain	IV or Stereotaxic injection	None
Gadolinium-DPTA	.2 mM/ml	0.05-0.2mmol/kg body weight; < 1ml for IV or IA < 1ul forstereotaxic	Rear Leg or Brain	IV, IA, or Stereotaxic	None
Manganese Chloride	30-120mM in isotonic pH-buffered Saline	Rats-up to 885umoles/kg body weight Mice-442umoles/kg body weight; Rats-2ml/hour Mice-0.25ml/hour	Rear Leg or Abdomen	IV or IP	hypothermia, dehydration
Manganese Chloride	3.9mmol/ml in isotonic pH-buffered Saline	1-4ul/mouse	Naris	Pipetted into the naris or at the base of the whiskers	Possible bleeding caused by tubing
Manganese Chloride	10mM in isotonic pH-buffered Saline	1ul. into specific areas of the brain; Max. dose is 0.4umoles/kg body weight	Brain- thalamus, ventricles, or cortex	Stereotaxic	None
Mannitol	25% Solution	5mg/kg body weight; single dose	Neck	IA	None-terminal experiment
Cereport (RNP-7)	9 ug/ml	9.0ug/kg body weight; single dose	Rear Leg	IV	None-terminal experiment
D-Amphetamine Sulfate	10 mg/ml	20mg/kg body weight	Rear Leg	IV	None-terminal experiment
Endothelial Growth Factor (EGF)	360 ng/ml	20-360ng	Brain ventricles	Stereotaxic	None
Nitric-Oxide Synthase (NOS) Inhibitors	Vary according to type; 1 mM solution in artificial CSF	Doses vary according to type used and mode of application; 30 mg/kg body weight, 40 mg/kg body weight	Rear Leg, Abdomen, Brain-exposed cortex	IP, IV, or Topical over exposed cortex of brain	None

Common Drugs for Use in Animals

*Common Drugs For Use in Animals

The following pages provide tables of drugs commonly used at the National Institutes of Health (NIH) for pre-anesthesia, anesthesia, analgesia, sedation, tranquilization, and restraint of laboratory animal species.

The dosage recommendations and other data presented on the following pages are based upon current data in the literature and the professional judgement of veterinarians.

Proper drug doses may vary greatly depending on species, strain, sex, age, physiologic status of the animal, and the level of anesthesia/analgesia desired.

Although these lists provide a ready source of information on drug doses, individuals should not use these drugs without prior experience.

Your institute or animal facility veterinarians are available for consultation and additional information.

The page facing each table provides species specific information.

Controlled drugs are identified by a "C." The Roman numeral classifies the drug into one of the five established schedules of controlled substances (e.g., sodium pentobarbital, C-II).
Abbreviations:
IV = intravenous
IM = intramuscular
IP = intraperitoneal
SC = subcutaneous
PO = per os, oral
IH = inhalation
qXh = every X hours

* The tables provided here are modified versions of the tables presented at http://oacu.od.nih.gov/ARAC/tablesbyspecies.pdf

SPECIES INFORMATION

MOUSE (*Mus musculus*)

Physiologic parameters:

Body temperature = 36.5-38.0ºC
Heart rate = 325-780/min
Respiratory rate = 94-163/min
Tidal volume = 0.09-0.23 ml

The use of chloroform as an anesthetic agent is discouraged. Chloroform can cause renal tubular calcification and/or necrosis, particularly in male mice; DBA/2 strain most susceptible.

Avertin is made by mixing equal amounts of tribromyl ethyl alcohol and tertiary amyl alcohol (2.5% dilution). If Avertin is improperly prepared or stored in the light, it will break down into dibromoacetic acid and hydrobromic acid which can be lethal in 24 hours. **Freshly mixed solutions are strongly recommended for safe use.** The solution can be kept as long as 4 months if it is stored in the dark at 4 degrees C. The solution should be tested to ensure that it has a pH >5.

* The therapeutic dose for carbon dioxide is close to the lethal dose; very short acting. Concurrent administration of 10-50% O_2 is recommended.

** Best for minor surgery procedures only.

MOUSE (*Mus musculus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.02-0.05 mg/kg	SC, IM
Diazepam, C-IV (Valium®)	5 mg/kg	IP
Ketamine, C-III (Ketaset®, Vetalar®)	22-44 mg/kg	IM
Carbon dioxide* + 10-50% O_2	To effect	IH
Anesthesia		
Sodium Pentobarbital, C-II	50-90 mg/kg	IP
Ketamine**, C-III	50-200 mg/kg	IP
Avertin (Tribromoethanol)	125-250 mg/kg 0.02 ml/g (1.2% solution)	IP
Ketamine/Xylazine:		
Ketamine	80-100 mg/kg Ketamine	IM, IP
Xylazine	5-7 mg/kg Xylazine	IM, IP
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	5-10 mg/kg q2-4h	SC IP
Butorphanol tartrate (Torbugesic®), C-IV	2.5-5 mg/kg q1-2h	SC
Buprenorphine, C-V	0.05-0.1 mg/kg q8h	SC IP
Oxymorphone, C-II	0.15 mg/kg q4h	IM
Ketoprofen	5 mg/kg q24h	SC

SPECIES INFORMATION

RAT (*Rattus norvegicus*)

Physiologic parameters:

Body temperature = 35.9-37.5°C
Heart rate = 250-450/min
Respiratory rate = 70-115/min
Tidal volume = 0.6-2.0 ml

Male rats and animals receiving low calorie diets require higher doses of barbiturates.

Avertin has been reported to cause ileus in rats

The therapeutic dose for carbon dioxide is close to the lethal dose; very short acting. Concurrent administration of 10-50% O_2 is recommended.

The reversal agent, yohimbine, is only effective when xylazine or medetomidine has been used.

* The projected duration of action for an analgesic is an approximation because the nature of the procedure and the level of pain that is experienced affect it.

RAT (*Rattus norvegicus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.04-0.1 mg/kg	SC
Diazepam, C-IV (Valium®)	5-15 mg/kg	SC
Ketamine, C-III (Ketaset®, Vetalar®)	22-50 mg/kg	IM
Carbon dioxide + 10-50% O_2	To effect	IH
Anesthesia		
Sodium Pentobarbital, C-II	30-60 mg/kg	IV IP
Ketamine, C-III (10 mg/ml solution)	50-100 mg/kg IP	IM
Ketamine/Xylazine:		
ketamine	60-80 mg/kg	IM
xylazine	5-7 mg/kg	IM
Halothane (Fluothane®)	To effect	IH
Isoflurane	To effect	IH
Carbon dioxide	To effect	IH
Telazol®, C-III	20-40 mg/kg	IP
	20 mg/kg	IM
Ketamine/Medetomidine		
ketamine	60-75 mg/kg	IP
Medetomidine (Domitor®)	0.25-0.5 mg/kg	SC
Analgesia*		
Morphine, C-II	1.5-3 mg/kg q2-4h	SC
Butorphanol tartrate, C-IV (Torbugesic®)	2.5-5 mg/kg q1-2h	SC
Ketoprofen	5 mg/kg q12h	SC
Buprenorphine, C-V	0.01-0.05 mg/kg	SC
Reversal Agents		
Yohimbine (reverses xylazine)	1-2 mg/kg	IM IP

SPECIES INFORMATION

HAMSTER (*Mesocricetus auratus*)

Physiologic parameters:

Body temperature = 37-38$_{o}$C
Heart rate = 250-500/min
Respiratory rate = 35-135/min
Tidal volume = 0.6-1.4 ml

Syrian or golden hamster is very resistant to morphine - no sedation or hypnotic effects.

Syrian or golden hamster has an increased tolerance to pentobarbital.

HAMSTER (*Mesocricetus auratus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	.1 mg/kg	IP IM SC
Ketamine, C-III (Ketaset®, Vetalar®)	20-60 mg/kg	IM
Diazepam	5 mg/kg	IP, IM
Anesthesia		
Sodium Pentobarbital, C-II	100-200 mg/kg	IP
Ketamine/Xylazine:		
Xylazine	7-10 mg/kg	IP IM
Ketamine	80 mg/kg	IP
Telazol®, C-III	20-80 mg/kg	IP IM
Isoflurane	To effect	IH
Analgesia		
Buprenorphine, C-V	0.05-0.1 mg/kg q8-12h	SC IM
Butorphanol tartrate, C-IV (Torbugesic®)	1-5 mg/kg q2-4h	SC IM

SPECIES INFORMATION

GUINEA PIG (*Cavia porcellus*)

Physiologic parameters:

Body temperature = 37.2-39.5°C
Heart rate = 230-380/min
Respiratory rate = 42-104/min
Tidal volume = 2.3-5.3 ml/kg

Large cecum can act as reservoir for anesthetics. Depending on drug solubility, the cecum can alter the pharmacologic effect.

Induction of anesthesia using volatile anesthetics (e.g., halothane and isoflurane) should be done with caution due to initial breath holding when animals are first exposed to irritating gas vapors.

Repeated exposure to halothane can cause hepatotoxicity. Isoflurane is a safer inhalant anesthetics to use.

Self mutilation has been reported in guinea pigs after ketamine administration.

GUINEA PIG (*Cavia porcellus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.05 mg/kg	SC
Diazepam, C-IV (Valium®)	2.5-5.0 mg/kg	IP IM
Acetylpromazine	5-10 mg/kg	M SC IV
Ketamine, C-III (Ketaset®, Vetalar®)	22-30 mg/kg	IM
Anesthesia		
Sodium Pentobarbital, C-II	15-40 mg/kg	IP
Sodium Thiopental, C-III	20 mg/kg	IV
Ketamine, C-III	40-50 mg/kg	IM
Ketamine/Xylazine:		
Xylazine	5 mg/kg	SC
Ketamine	30-40 mg/kg	SC
Isoflurane	To effect	IH
Analgesia		
Buprenorphine, C-V	.01-0.05 mg/kg q8-12h	SC
Morphine, C-II	10 mg/kg q2-4h	SC IM
Butorphanol tartrate, C-IV (Torbugesic®)	0.25-0.4 mg/kg	IV SC
Reversal Agent:		
Atipemazole (Antisedan®)	1 mg/kg	IM IV SC IP

SPECIES INFORMATION

CHINCILLA *(Chinchilla laniger)*

Physiologic parameters:

Body temperature = 38-39 C
Heart rate = 100-150 min
Respiratory rate = 40-80 min

Chinchillas are extremely susceptible to stress, all surgical procedures should be undertaken with caution. Premedication should be utilized for all surgeries.

CHINCILLA *(Chinchilla laniger)*

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.5 mg/kg	IM
Acepromazine	0.5 mg/kg	IM
Valium	3.0-5.0 mg/kg	IM
Anesthesia		
Ketamine/Acepromazine:		
Acepromazine	0.5 mg/kg	IM
Ketamine	40 mg/kg	IM
Ketamine/Xylazine:		
Ketamine	40 mg/kg	IM
Xylazine	5 mg/kg	IM
Ketamine/Valium		
Valium	1.0-2.0 mg/kg	IM
Ketamine	20-40 mg/kg	IM
Pentobarbital	30 mg/kg	IV
	40 mg/kg	IP
Isoflurane	To effect	IH
Analgesia		
Consult your veterinarian		

SPECIES INFORMATION

Ground Squirrel **(to be completed)**

SPECIES INFORMATION

RABBIT (*Oryctolagus cuniculus*)

Physiologic parameters:

Body temperature = 38-39.6°C
Heart rate = 130-325/min
Respiratory rate = 32-60/min
Tidal volume = 4-6 ml/kg

Many rabbits have serum atropinesterase which causes reduced response to atropine. Glycopyrrolate, another anticholinergic, can be used instead of atropine.

Unique hypnotism or immobilization reflex has been observed in rabbits in the absence of drug use.

Large cecum can act as reservoir for anesthetics. Depending on drug solubility, the cecum can alter the pharmacologic effect.

Induction of anesthesia using volatile anesthetics (e.g., halothane and isoflurane) should be done with caution due to initial breath holding when animals are first exposed to irritating gas vapors.

Give IV injections via marginal ear veins.

Self mutilation has been reported in rabbits after IM ketamine administration. Dilution of ketamine with saline will limit this side effect.

RABBIT (*Oryctolagus cuniculus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.04-0.5 mg/kg	SC IM
Ketamine, C-III (Ketaset®, Vetalar®)	15-50 mg/kg	IM
Acetylpromazine (Acepromazine)	1.0-10 mg/kg	IM SC IV
Ketamine/Acetylpromazine (10:1)	15-50 mg/kg	IM
Diazepam, C-IV (Valium®)	5-10 mg/kg	IV IM
Glycopyrrolate	0.005-0.011 mg/kg	IM
Butorphanol & Acepromazine		
Butorphanol tartrate, C-IV (Torbugesic®)	1 mg/kg	SC
Acetylpromazine	1 mg/kg	SC
Anesthesia		
Sodium Pentobarbital, C-II (3% solution given slowly to effect)	15-40 mg/kg	IV
Ketamine/Xylazine/Acepromazine:		
Xylazine	5-10 mg/kg	IM
Ketamine, C-III	35-50 mg/kg	IM
Acepromazine	0.75 mg/kg	IM
Ketamine/Midazolam		
Ketamine, C-III	25 mg/kg	IM
Midazolam, C-IV	1 mg/kg	IM
Ketamine/Diazepam		
Ketamine, C-III	15-50 mg/kg	IM
Diazepam, C-IV	5-10 mg/kg	IM
Ketamine/Acepromazine/Butorphanol		
Ketamine, C-III	35 mg/kg	SC
Acepromazine	0.75 mg/kg	SC
Butorphanol tartrate, C-IV (Torbugesic®)	0.1 mg/kg	SC
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	2-5 mg/kg q2-4h	SC IM
Buprenorphine, C-V	0.02-0.1 mg/kg q8-12h	SC
Butorphanol tartrate, C-IV (Torbugesic®)	0.1-1.5 mg/kg q4h	IV
	1.0-7.5 mg/kg q4h	IM SC
Flunixin meglumine (Banamine®)	1.1 mg/kg q12h	IM SC
Carprofen	1.5 mg/kg q12h	PO
Ketoprofen	3 mg/kg q12h	IM
Reversal Agents		
Yohimbine (reverses xylazine)	0.2 mg/kg	IV
Doxapram (all anesthetics)	5-10 mg/kg	IM, IV, IP

SPECIES INFORMATION

NONHUMAN PRIMATES

Physiologic parameters:

Rhesus	Baboon
Body temperature = 37-39$_{o}$C	Body temperature = 39$_{o}$C
Heart rate = 120-180/min	Heart rate = 150/min
Respiratory rate = 32-50/min	Respiratory rate = 35/min
Tidal volume = 21 ml	Tidal volume = 50 ml

The dosage and frequency of administration of all analgesic agents must be tailored to the animal, procedure, and magnitude of pain present. Combinations of narcotics and non-steroidal agents are commonly used. Consult your veterinarian for specific recommendations.

* Pre-medication with Atropine or Glycopyrrolate is suggested to avoid bradycardia and cardiac arrhythmias with these agents.

** Poor analgesia. Adequate for superficial procedures only!

NONHUMAN PRIMATES

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.02-0.05 mg/kg	IM SC
Glycopyrrolate	0.005-0.01mg/kg	IM SC
Diazepam, C-IV (Valium®)	0.5-1.0 mg/kg	IM
Xylazine	0.5-2.0 mg/kg	IM
Anesthesia		
Sodium Pentobarbital, C-II	20-30 mg/kg	IV
Sodium Thiopental, C-III (2.5%)	15-20 mg/kg	IV
Ketamine/Xylazine*:		
Ketamine, C-III	7-10 mg/kg	IM
Xylazine (Rompun®)	0.25-2.0 mg/kg	IM
Ketamine/Diazepam**:		
Ketamine, C-III	5 mg/kg	IV
Diazepam, C-IV (Valium®)	1 mg/kg	IV
Ketamine/Midazolam**:		
Ketamine, C-III	15 mg/kg	IV
Midazolam, C-IV	0.5-0.15 mg/kg	IV
Telazol®, C-III	4.0-6.0 mg/kg	IM
Halothane (Fluothane®)	To effect	IH
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	1-2 mg/kg q4h	IM SC
Oxymorphone, C-II	0.15 mg/kg q4-6h	IM
Buprenorphine, C-V	0.01-0.03 mg/kg q8-12h	IM SC
Acetylsalicytic Acid (Aspirin)	10-20 mg/kg q6h	PO
Acetaminophen	10 mg/kg q8h	PO
Flunixin meglumine (Banamine®)	0.5 mg/kg daily	IM
Butorphanol tartrate, C-IV (Torbugesic®)	0.025 mg/kg q3-6h	IM
Naproxen	10 mg/kg q12h	PO
Ketorolac	15-30 mg/kg	IM
Reversal Agents		
Yohimbine (reverses xylazine)	0.05 mg/kg	IV
Naloxone (reverses opioids)	.1-0.2 mg/kg as needed	IV

AMPHIBIANS

Anesthesia

Amphibians must be kept moist over their entire bodies during anesthesia and recovery. Care must be taken that they do not become immersed, as this will result in drowning.

Tricaine (MS 222) -ethyl m-amino benzoate methanesulfonate (tricaine methane sulfonate) Should be buffered to neutral pH before use. MS222 must be disposed as chemical waste.

Immerse in water with agent added:
1:2000 to 1:1000 for adults (i.e.,5-10mg of tricaine in 1000 ml water)
1:3000 to 1:5000 for larvae

Induction in 5-20 minutes; maintain by moist cloth contact with MS 222 solution.

Recovery - keep at 22-26$_{o}$C; takes 3-6 hours; keep moist.

Benzocaine 100 mg/1000 ml water

Halothane/Isoflurane - 5% in anesthetic chamber; maintain at 3%.

Sodium Pentobarbital- 60 mg/kg; inject into dorsal lymph sac.

Analgesia

Chlorpromazine	32 mg/kg; inject into dorsal lymph sac
Chlordiazepoxide	90 mg/kg; inject into dorsal lymph sac
Buprenorphine, C-V	14 mg/kg; inject into dorsal lymph sac
Diphenhydramine	51 mg/kg; inject into dorsal lymph sac

FISH

Because fish breathe through gills rather than lungs, anesthesia must be delivered through an aquatic medium. Most fish induced by adding the anesthetic agent to the tank water. It is important to have two separate tanks; one for anesthesia and one for recovery. Water for anesthesia should be well-aerated to provide adequate oxygen and minimize the stress of induction. Food should be withheld for several hours prior to induction.

Tricaine (MS 222) -ethyl m-amino benzoate methanesulfonate (tricaine methane sulfonate)
Should be buffered to neutral pH before use. MS222 must be disposed as chemical waste.

Immerse in water with agent added; doses vary according to species:
1:20,000 (50 mg/liter) for tranquilization
1:10,000 (100 mg/liter) for surgical anesthesia

Induction occurs within 3 minutes, recovery takes 10-15 minutes after removal.

Benzocaine 20-30 mg/1000 ml water for tranquilization
50 mg/1000 ml water for surgical anesthesia

Etomidate is an analog of propoxate and provides sedation only. It should not be used for procedures requiring surgical anesthesia.

0.05 -0.5 mg/1000 ml for tranquilization during transportation
2-4 mg/1000 ml for sedation

Animal Health and Care Section (AHCS) SOP 301: Maintenance of Guillotines

ANIMAL HEALTH AND CARE SECTION
NINDS

SOP 301
August 1998

SUBJECT: MAINTENANCE OF GUILLOTINES

A. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to ensure that guillotines are kept in working order. The blade movement should be smooth with no perceptible binding or resistance. The blade must be rust-free, clean, sharp and decapitate with minimal force.

B. APPLICABILITY

The contents of this SOP apply to all personnel in the NINDS/NIDCD who have approved animal studies authorizing the use of a guillotine.

C. GENERAL INFORMATION

1. The guillotines should be marked with a number or other identification.

2. Guillotines should not be moved from room to room. If you need to transfer a guillotine to a different room, the guillotine should be sanitized before moving and before returning to the original room.

3. Log books for guillotines are located in each procedure room and these books should be notated with each use. In laboratories, a log book should be located near the guillotine and each use should be notated.

D. RESPONSIBILITIES

1. Anyone using a guillotine should ensure it is in good condition prior to its use. If a guillotine in the animal facility is not working properly, please report this to the Floor Leader or Facility Manager (301-451-0978, or 301-451-0980, or 301-496-7108) so it can be repaired.
2. Personnel using a guillotine are responsible for proper cleaning after use.
3. The Floor Leader will ensure that the animal facility guillotines are lubricated as needed with silicon.
4. Investigators may contact the Floor Leader or Facility Manager if they want their guillotine lubricated by AHCS staff.

5. The Floor Leader/Facility Manager will ensure that facility guillotines are rotated for sharpening at a minimum of every twelve months or more often if needed. Depending on species and number of animals, investigators may need to have the blades on their laboratory guillotines sharpened every six months.

6. Persons responsible for guillotine(s) must maintain a log book. The log book should include the following information:

    a) Identification number of the guillotine;

    b) Room location;

    c) Person responsible for maintenance and repair;

    d) Usage tracking (i.e. date, species and number of animals euthanized);

    e) Date of blade sanitization and sharpening (to be done annually or more frequently if use requires).

E. PROCEDURE

1. Daily Use

    a) Before using the guillotine, check for rust, blade sharpness, ease of blade movement and cleanliness.

    b) After use, rinse the entire guillotine under fast-running cold water to remove any blood and tissues.

    c) The base should be carefully scrubbed with disinfectant to reduce gross contamination.

    d) A final alcohol rinse will assure evaporation and reduce the need to hand-dry the equipment. The guillotine should be turned upside down with the blades opened to facilitate drying.

2. Maintenance

    a) The Animal Health and Care Section (AHCS) will maintain the guillotines in NINDS Managed Animal Facilities.

    b) If an investigator needs to have a guillotine sanitized and/or blade sharpened, the AHCS will assist in this service. **All blades must be sharpened annually, as a minimum.**

c) Bring the guillotine to be serviced to AHCS Floor Leader/Facility Manager and provide a CAN number for billing of sharpening service.

d) If an investigator needs a replacement guillotine while their guillotine is being sharpened, contact Floor Leader/Facility Manager to receive a temporary replacement.

e) Only qualified people should take a guillotine apart. The Floor Leader/Facility Manager can provide assistance.

f) Prior to sharpening or more often if needed, the guillotine should be taken apart and sanitized by running the guillotine through the tunnel washer in a basket. Contact the Floor Leader/Facility Manager to request sanitization.

1) Frequency of sharpening depends on both frequency of use and the species euthanized.

2) For example, using the guillotine only 1-2 times per month may require less frequent sharpening than heavy use (many animals, 4-6 times per month).

3) Species will also influence how often blades should be sharpened. For example, 10-20 mice euthanized 2-3 times per month may require less frequent sharpening of blades than 5-10 guinea pigs 2 times per month.

4) In short, use common sense. The best cutting blade dulls after use.

g) After sanitizing, the guillotine is taken to BEIP, Bldg.13, 3rd Floor, room 3W24 ATTENTION: Mr. Jim Powell, 301-594-3460 for sharpening.

h) The sharpening should take no longer than two weeks. Contact Mr. Powell if you have not been notified to pick up the guillotine after two weeks.

4. Replacement

a) Mr. Powell and his staff will determine if the blades can be sharpened or should be replaced.

b) If the blades cannot be sharpened, the guillotine should be taken apart and the blades disposed of in a sharps container; place the stand in the dumpster, NOT MPW.

5. The NINDS/NIDCD Animal Care and Use Committee (ACUC) will ask to review your guillotine log book during the semi-annual review of labs, animal holding facilities, and program review.

F. POINTS OF CONTACT

Facility Manager	301-451-0978, or 301-496-7108
Floor Leader	301-451-0980
Administrative Tech	301-496-8488